REMARKS

Reconsideration is requested.

Claims 2-25 are pending. Claim 5 has been allowed. The indication of allowable subject matter in claims 3-4, 11, 13 and 15, is acknowledged, with appreciation.

Withdrawal of the restriction requirement is acknowledged, with appreciation.

The inclusion on a PTO 892 Form of the references originally listed on the applicants PTO 1449 Forms filed February 2, 2001 (i.e., the Examiner's references U-1 and X-1, which were lined-through in the PTO 1449 Form returned with the Office Action dated October 15, 2002) and April 15, 2003 (i.e., the Examiner's references V-1 and W-1, which were listed as the applicants doc. Nos. 8 and 9, respectively, and lined-through in the initialed PTO 1449 Form returned with the Office Action dated October 24, 2003) is acknowledged, with appreciation. The Examiner has also listed the reference designated as "X-1" in the PTO 892 Form returned with the Office Action of March 16, 2005 as reference "V" in the PTO 892 Form returned with the Office Action of October 15, 2002.

Acceptance of the drawings is acknowledged, with appreciation.

The specification has been amended above as may be required by Rule 78.

The Section 112, second paragraph, rejection of claims 6-9, 12 and 19-21, is obviated by the above amendments. Withdrawal of the rejection is requested.

Specifically, claims 6-9 and 20-21 have been amended to recite "coding for" as the Examiner's preferred word choice. The metes and bounds of the unamended claims would have been appreciated by one of ordinary skill in the art. Claim 12 has been amended to refer to claim 6, as suggested by the Examiner. The Examiner's

helpful suggestion in the regard is noted, with appreciation. Claim 19 has been amended to advance prosecution, without prejudice. None of these amendments are intended to narrow the scope of the amended claims; and these amendments are not being made for substantial reasons relating to patentability.

Withdrawal of the Section 112, second paragraph, rejection is requested.

The Section 112, first paragraph "enablement", rejection of claims 2,6, 12, 14 and 16-25 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following comments.

The applicants submit that uricase enzymes, from bacterial, yeast and mammalian sources, are well known. The art cited by the Examiner and further discussed below, is evidence of the same. Moreover, the wealth of art made of record by the applicants is evidence that one of ordinary skill in the art at the time of the present invention was well versed in manipulating a variety of uricase proteins and assessing uricase activity both *in vitro* and *in vivo*.

The Examiner's acknowledgement that the present application is enabling for the specific exemplified species of SEQ ID NOs: 1-4 and 8-11 is acknowledged, with appreciation. The Examiner has therefore appreciated that the applicants have taught how to make and use at least six (6) different species within the claimed genus. Moreover, as claims 3 and 4 have not been rejected as allegedly not being supported by an enabling disclosure, the Examiner has confirmed that the subgenuses of claims 3 and 4[1] are supported by an enabling disclosure.

[1] Claim 3. A protein of claim 2 wherein said recombinant uricase chimeric protein comprises 304 amino acids, the first 225 N-terminal portion of said 304 amino acids being amino acids 1-225 of porcine

The applicants respectfully submit that one of ordinary skill who is able to make and use the subject matter of the subgenuses of claims 3 and 4 would be able to make and use the subject matter of claims 2, 16 and 17, as well as the other rejected claims, without an undue amount of experimentation.

The Examiner's concluding comments relating to claims 16 and 17 are noted however the Examiner is urged to appreciate that claims 16 and 17 require modification of a uricase protein, which proteins are well known to those of ordinary skill. Moreover, claim 17 requires introduction of a lysine in the place of an arginine.

Withdrawal of the Section 112, first paragraph, rejection of claims 2, 6, 12, 14 and 16-25, is requested.

The Section 103 rejection of claims 16 and 17 over either Chen (Biochimica et Biophysica Acta, 660 (1981) 293-298), Chua (Annals of Internal Medicine, 15 July 1988, 114-117), Davis (the Lancet, August 8, 1981, 281-283), Savoca (Int. Archs Allergy appl. Immun. 75: 58-67 (1984)), or Nishimura (Enzyme 26: 49-53 (1981) in view of Hershield (PNAS 88, 7185-7189, August 1991) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing remarks.

The Examiner asserts that the primary references, i.e., Chen, Chua, Davis , Savoca and Nishimura, each teach the modification of uricase with PEG, with non-deleterious results. See, page 4 of the Office Action dated March 16, 2005.

The Examiner also asserts that Hershfield teaches that

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uricase and the remaining 79 amino acids of said 304 amino acids being amino acids 226-304 of baboon uricase.

Claim 4. A protein of claim 2 wherein said recombinant uricase chimeric protein comprises 304 amino acids, the first 288 N-terminal portion of said 304 amino acids being amino acids 1-288 of porcine

> "PEG can reduce the immunogenicity and prolong the circulating life of enzymes and that more PEG can be introduced into an enzyme by replacing Arg with Lys." Id.

The Examiner has concluded that it would have allegedly be obvious for one of ordinary skill in the art to have used the teachings of any of the primary references

> "that non-deleterious PEG can be introduced into uricase along with the teaching of Hershfield that more PEG can be introduced into enzymes by replacing Arg with Lys to replace Arg with Lys residues with the expectation that the modified enzyme molecules would have PEG introduced at non-deleterious sites." Id.

Consideration of the following in response is requested.

Initially, the applicants note that the invention of claims 16 and 17 relate to methods of increasing the available non-deleterious PEG attachment sites in a mammalian uricase protein. The Examiner's secondary reference, i.e., Hershfield, relates to modification of *E. coli* purine nucleoside phosphorylase (PNP) as opposed to mammalian uricase.

Hershfield further describes that the introduction of PEG attachment sites in to PNP to "more effectively mask" residual epitopes

> "assumes that arginine residues are also likely to occur on the protein surface and that many Arg – Lys substitutions will not have severe adverse effects on enzyme folding, subunit assembly, stability, or function." See, page 7189, left column, lines 12-15 of Hershfield.

There is no teaching or suggestion in Hershfield that mammalian uricase would similarly be expected to occur on the protein surface and not have an adverse effect on enzyme folding or function, for example.

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uricase and the remaining 16 amino acids of said 304 amino acids being amino acids 289-304 of baboon uricase.

Moreover, Hershfield states that while residual immunogenicity and antigenicity of PEG-modified wild-type *E. coli* PNP had been reduced, it was unclear whether immunogenicity was reduced sufficiently to allow treatment of human PNP deficiency with PEG modified RK2 PNP. Id. 2nd full paragraph. Accordingly, Hershield expresses at least some doubt that the limited mice studies presented therein are predictive of treatment utility.

More importantly perhaps, Hershield teaches that immunogenicity is not the only concern in developing enzyme or other protein-based therapies. Rather, Hershfield teaches that other factors

> "such as stability, specific activity, or substrate range, may be of comparable or overriding importance, as illustrated by the contrasting properties of bacterial and mammalian PNP." Id. Passage spanning right and left columns.

There is no teaching in Hershfield regarding uricase enzymes. Moreover, one of ordinary skill in the art would presume, at best, from Hershfield that similar "other factors" discussed by Hershield with regard to PNP would need consideration with regard to uricase.

As for the combination of the teachings of each primary reference with Hershfield, consideration of the following is requested.

Chen teaches that PEGylated yeast and hog liver uricase have a decreased uricase enzyme activity in a direct relation to the amount of PEGylation. Specifically, the uricase enzyme activity of yeast uricase containing PEG attached to 57% of the amino groups retained 23% of the original enzyme activity and uricase activity of hog

liver uricase containing PEG attached to 58% of the amino groups retained 28% of the original activity, while "appearing" to be non-immunogenic. Chen reports that

> "Other preparations of poly(ethylene glycol):urate oxidase were made using smaller ratios of the activated-poly(ethylene glycol) to amines. Although more active, these preparations showed evidence of immunogenicity." See, page 294, right column, second full paragraph, of Chen.

Accordingly, Chen describes a balance between enzyme activity and amount of PEGylation which would have suggested to one of ordinary skill that increasing the PEGylation beyond the 57-58% amount taught by Chen, would decrease the enzyme activity to an unacceptable level. Of interest also is Chen's teaching that PEGylation of 37 and 47% hog liver uricase was unable to maintain the longer circulating lives in mice (Figure 2 of the reference) whereas the 58% modified level was able to maintain the longer circulating lives over the 90 days tested (Figure 3 of the reference).

Contrary to the Examiner's assertion therefore, there was motivation in Chen to not increase the amount of PEGylation, or PEG attachment sites, in uricase as to do so would have been expected to have further reduced the amount of enzymatic activity. There is no motivation in Chen or Hershfield therefore to combine the references to produce the invention of either claims 16 or 17 of the present application.

Withdrawal of the Section 103 rejection of claims 16 and 17 over Chen and Hershfield is requested.

Chua reports on the experience in treating hyperuricemia in a single patient with Non-Hodgkin Lymphoma with the administration o PEG-modified *Arthrobacter protoformae* uricase supplied by Enzo Inc. Chua does not relate to mammalian uricase proteins or to methods of modifying mammalian uricase proteins to increase the

available non-deleterious PEG attachment sites, as presently claimed. In fact, Chua teaches a reduction in serum uricase level by the third injection and an absence of the sharp fall in urate observed after the second injection. See, Figure 1 and Results Section of Chua. Chua theorize that the rise in uricase within 2.5 hours of the second injection of PEG-uricase suggests that some of the dose may have been inadvertently administered intravenously. See, page 116, left column, first full paragraph of Chua.

Chua is submitted to be a report of an uncontrolled treatment of a single ill individual with a non-mammalian uricase of unknown PEGylation amount or degree. It is unclear what one of ordinary skill in the art would have produced from a combination of Chua and Hershfield as Chua does not suggest that increasing the amount of PEGylation would have overcome the problems noted in Chua or increased the activity of the uricase in Chua or otherwise changed the results or experiences reported in Chua.

Moreover, there is no suggestion in Chua that the single patient treated in Chua produced antibodies or produced an immune response against the *Arthrobacter* uricase such that there would not have been any motivation to combine the teachings of Hershfield (i.e., to reduce immunogenicity).

Withdrawal of the Section 103 rejection of claims 16 and 17 over Chua and Hershfield is requested.

Davis teaches the PEGylation of 71% of the amino groups in yeast uricase to produce a preparation retaining only 11% of the enzyme activity. These results are similar to the trend suggested by Chen, as discussed above, wherein increasing the amount of PEGylation decreases the remaining enzyme activity. Davis does not relate

to mammalian uricase proteins or to methods of modifying mammalian uricase proteins to increase the available non-deleterious PEG attachment sites, as presently claimed.

Davis teaches that the PEGylated yeast uricase was non-immunogenic and resulted in a rapid disappearance of serum uric acid for over 32 hours in five (5) men with advanced haematological malignancies. One of ordinary skill in the art would not have further modified the uricase of Davis, to provide more PEG attachment sites, as the Examiner interprets Hershfield to teach, as to do so would likely have reduced the enzyme activity even further. Moreover, the composition of Davis is described as being non-immunogenic such that the motivation of Hershfield would not have motivated one of ordinary skill in the art to have altered Davis to make the present invention of claims 16 and 17.

Withdrawal of the Section 103 rejection of claims 16 and 17 over Davis and Hershfield is requested.

Savoca, like Davis, relates to PEGylated yeast uricase. Savoca specifically relates to induction tolerance of mice by PEGylated yeast uricase. Savoca reports that yeast uricase with PEG attached to 35% of the available amino groups behaves as a weak immunogen, and that yeast uricase with PEG attached to 70% of the available amino groups is non-immunogenic and nonantigenic. See, page 61 of Savoca. These results are consistent with the teachings of Davis and Chen, for example, discussed above. Savoca concludes however that yeast uricase with PEG attached to 35% of the available amino groups

> "was found to induce the most effective tolerance in both unsensitized and sensitized mice." See, Abstract, last sentence, of Savoca.

Accordingly, Savoca concluded that a decreased amount of PEGylation (i.e., 35% versus 70%) was optimal. This conclusion teaches away from the Examiner's conclusion that one of ordinary skill in the art would have been motivated by the cited art to introduce more PEG into uricase.

Savoca does not relate to mammalian uricase proteins or to methods of modifying mammalian uricase proteins to increase the available non-deleterious PEG attachment sites, as presently claimed. To the contrary, Savoca, if anything, would have motivated one of ordinary skill in the art to have reduced the number of PEG attachment sites in yeast uricase to induce the most effective tolerance in both unsensitized and sensitized mice. Combination of Hershfield with Savoca in the manner suggested by the Examiner would have been contrary to the conclusions of Savoca.

Withdrawal of the Section 103 rejection of claims 16 and 17 over Savoca and Hershfield is requested.

Nishimura, like Davis and Chen and Savoca, relates to PEGylation of yeast uricase. Moreover, Nishimura confirms that increasing the degree or amount of PEGylation reduces enzymatic activity. See Table I of Nishimura. The comments above with regard to Davis and Chen and Savoca therefore are relevant to the lack of motivation in the cited art to have combined Nishimura with Hershfield to have made the invention of claims 16 and/or 17 of the present application.

Nishimura does not relate to mammalian uricase proteins or to methods of modifying mammalian uricase proteins to increase the available non-deleterious PEG attachment sites, as presently claimed.

Withdrawal of the Section 103 rejection of claims 16 and 17 over Nishimura and Hershfield is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned in the event anything further is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

B. J. Sadoff
Reg. No. 36,663

BJS:
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100